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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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ABSTRACT

In collaboration with the personnel at The University of Michigan, we have developed two types of arrays of thin-film microprobes suitable for chronic implantation into the cat's cortex, and for prolonged intracortical microstimulation . The arrays have 8 or 16 iridium sites with geometric surface areas of 400 μm^2. The arrays include a long (2.5 cm) horizontal integrated cable, which we reinforce by coating both sides with MDX4210 silicon elastomer. The assembly includes an implantable junction box between the integrated cable and the longer Teflon-insulated leads to the percutaneous connector. These assemblies are mechanically and electrically stable for at least 33 days (our longest experience to date). At 33 days after implantation, the microelectrodes are capable of evoking responses in the corticospinal (pyramidal) tract with thresholds comparable to those evoked by discrete iridium intracortical electrodes (less than 10 μA). They also allow recording of the action potentials from individual neurons, with an acceptable signal-to-noise ratio.

There are some remaining problems relating to positional stability during prolonged residence in in the brain, and to electrical stability during prolonged pulsing. The arrays have a sharp spine (superstructure) , and threfore have a tendency to sink into the cortex. We have modified the array to include a silicon cross-brace, to help to support the array on the surface of the pia.

After pulsing the electrode sites in two cats (IC144 and IC146) for 7 hours with biphasic, cathodic-first current pulses, 400 μsec/phase in duration and 20 μA in amplitude (8 nC/phase, 5 A/cm^2, 2000 $\mu C/cm^2$), there was an accumulation of lymphocytes around the pulsed sites. In another animal (ic146), two of the electrodes sites failed (became electrically open) during the 7-hour stimulation, after remaining functional in vivo for 33 days. These results suggest that a stimulus charge per phase of 8 nC is excessive for these particular electrodes sites.

INTRODUCTION

These studies are part of our investigations of the limits of safe microstimulation of neurons in the brain, and our investigations of the mechanisms underlying the neural damage and dysfunction that may result from surgical implantation and long-term residence of these electrodes in nervous tissue. The studies are utilizing discrete (activated iridium) microelectrodes and multisite silicon microprobes implanted chronically in the cerebral cortex of cats. In this report, we describe our first experience with chronically-implanted, electrically active, multisite silicon arrays

METHODS

Multisite thin-film silicon electrode arrays were designed in collaboration with Jamille Hetke at the University of Michigan. Two types of array were developed, one with 2 tines and 8 stimulating sites, and one with 4 tines and 16 stimulating sites (Figure 1A). The 4-tine probe was used in the studies described here. The arrays have horizontal integrated cables, 2.5 cm in length, that terminate in a bonding pad. The iridium electrode sites have geometric areas of 400 μm^2. The tips of the tines are formed by a shallow - diffusion boron etch-stop process, which yields tines with very sharp- edged shanks above the tip, and a very sharp tip.

The first step in the fabrication of the chronic implant assemblies is to coat both sides of the flexible integrated silicon cable with MDX4210 silicone elastomer. This strengthens the cable, while preserving most of its flexibility. The bonding pad region is then secured to a 16-pin chip carrier using a thin layer of marine epoxy, as shown in Figure 1B. A slot has been cut in one end of the chip carrier to accommodate the silicon cable. The pad-bonding sites on the chip are then bonded with gold wire to the inner conductor sites on the chip carrier using a semiconductor chip bonder. Teflon-insulated platinum iridium leads from the percutaneous connector are then bonded to the outer conductor on the chip carrier using silver solder, and the carrier is encapsulated in MDX4210 elastomer. The uncured elastomer is stabilized by a "shoe" of thin (.007") silicon rubber sheeting drawn up around the carrier. A support beam composed of stainless steel

wire is then attached to the top of this Implantable junction box. The beam supports a fine silk suture by which the silicon cable and array is drawn into the plane of the bonding pad. The 90° bend so formed allows the integrated cable to retain flexibility in two dimensions, although it has been twisted 90° about its axis. The complete junction box, cable and array are shown in Figure 1C. The assembly shown in Figure 1C incorporates a short silicone rubber crossbeam attached just proximal to the array, to reduce the tendency of the array to sink into the cortex (see below).

The iridium sites are then activated by potentiodynamic cycling, and the entire assembly is soaked for 4 days in deionized water, then sterilized with ethylene oxide prior to implantation.

Using general anaesthesia and aseptic surgical technique, the arrays are implanted into the precruciate (motor) cortex of adult cats. The intended clinical use of these arrays is as part of a visual prosthesis wherein they will be implanted into the primary visual cortex. However, the corticospinal projection (pyramidal tract) from the sensorimotor cortex provides a means of monitoring the effects of prolonged stimulation on the electrical excitability of cortical neurons, and for this reason, we implant the arrays into the cat's sensorimotor cortex. The scalp and muscles are reflected in a midline incision, and the cruciate (sensorimotor) cortex is exposed. The frontal air sinus is filled with bone cement. The cable junction box is attached with bone cement to the parietal bone, lateral and posterior to the craniectomy deficit. The suture suspending the array from the support beam is released, and, using padded forceps, the array of microelectrodes is inserted into the cortex through a small slit in the dura. The silicon cable remains above the dura.

To assist in the implantation of the recording electrode into the pyramidal tract, a surface stimulating electrode, 1 mm in diameter, is placed on the motor cortex adjacent to the microstimulating array. The surface array can excite a large number of corticospinal neurons, and this induces a large compound action potential (CAP) in the pyramidal tract. The flexible stainless steel pyramidal tract recording electrode is inserted by stereotaxis through a small burr hole over the cerebellum, to region of the track in the ventral aspect of the brainstem. Its final position in the ventral brainstem is adjusted until

the compound action potential from the pyramidal tract is maximized. The lead to the recording electrode is then secured to the posterior fossa with methyl methacrylate bone cement. The sensorimotor cortex is then covered with a thin layer of fibrin glue, then with a layer of gelfoam, and finally, the craniectomy defect is sealed with methacrylate bone cement. The cats are placed in an incubator where they are monitored until they have recovered from the anesthesia. They are given appropriate postoperative care, including Bupronorphine for relief of postoperative pain.

The electrical integrity of the microelectrodes was monitored approximately every two weeks. In the two experiments completed to date, the prolonged stimulation protocols were conducted 28 or 33 days after implantation. The stimulus was biphasic, cathodic-first current pulses, 400 μsec/phase in duration and 20 μA in amplitude (8 nC/phase, 5 A/cm^2, 2000 μC/cm^2). Our contract specified that the surface area of the electrodes sites should not be greater than 4×10^{-6} cm^2, which set some restriction on the range of stimulus parameters. Thus, a stimulus pulse amplitude of 20 μA corresponds to a current density of 5 A/cm^2, which is actually greater than the maximum current density of 4 Amps/cm^2 recommended for long-term pulsing of activated iridium microelectrodes (Robblee, personal communication). If the current density at the electrode surface is excessive, iridium oxide will form continuously, eventually destroying the microelectrodes.

Within 2 hours after the end of the 7-hour test stimulation, the cats were perfused for histologic evaluation of the implant sites. A brief saline rinse is perfused through the ascending aorta, followed by 2L of Karnovsky's fixative (3% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4). This fixative can be used for both light and electron microscopy of brain tissue. Perfusion is effected at 120 mm Hg, using a peristaltic pump (Model #7520-25 Cole-Palmer Instrument Co., Chicago, IL).

Following perfusion, the head with brain and array(s) in situ, is left in fixative until autopsy the following day. At autopsy the arrays are removed and examined with a dissecting microscope for evidence of adherent brain tissue, then stored in absolute ethanol for future scanning electron microscopy of the electrode tip. The array sites in the brain are examined for evidence of hemorrhage, infection, tissue compression and

connective tissue formation around the array matrix. Photographs are taken of the implant sites before and after removal of the arrays.

Tissue samples designated for light microscopy are resected as a single block containing all electrode tracks. Serial paraffin sections are stained with Nissl (for assessment of neural elements), or with Hematoxylin and Eosin (a nuclear stain used to identify the cell types of any inflammatory infiltrates). A few sections are stained with Masson's trichrome, to highlight connective tissue (scars)

Special attention is given to the distribution and extent of neural damage due to mechanical and/or electrical factors, and the presence of microvacuoles and microhematomas that arise from injury to the blood vessels of the parenchyma. The microvasculature is examined to determine the extent of neo-vascularization.

RESULTS

To date, prolonged stimulation protocols have been conducted in two cats implanted with thin-film arrays (IC-144 and IC-146). Eight of the 16 electrode sites were pulsed continuously and simultaneously for 7 hours at 100 pulses/sec. In cat IC146, the averaged compound action potentials (AECAPS) evoked from 3 or 4 of the microelectrode sites were recorded from the pyramidal tract before and after the 7 hours of stimulation. In cat IC144, the microelectrode array was inserted into the lateral aspect of the post cruciate gyrus in order to avoid a large blood vessel on the precruciate gyrus. Therefore, satisfactory pyramidal tract responses were not obtained from that animal.

Cat IC-144.

The microelectrode array was inserted into the lateral postcruciate gyrus so no AECAPS were recorded from the pyramidal tract. Figure 2A shows a histological section through the site of the spine of the intracortical array (scale bar = 250 μm). The array had sunk into the molecular layer of the cortex, to the extent of becoming partly buried in the tissue. There are degenerative changes in the molecular layer of the cortex, probably due to pressure from the knifelike spine of the array. In the next animal (IC146), the microelectrodes were inserted through a smaller slit in the dura, in an effort to reduce the compression of the cortex by the spine of the array. As noted below, this precaution

reduced, but did not eliminate, the tendency for the spine of the array to sink into the cortex. Therefore, arrays implanted in the future will include a short silicone rubber crossbeam to help support it on the surface of the pia (see Figure 1).

Figure 2B shows the track (T) left by one of the four intracortical tines (scale bar = 100 μm). There is an aggregate of lymphocytes adjacent to the tine, near one of the pulsed electrode sites. More lymphocytes can be seen surrounding the nearby small blood vessels (V) ("perivascular cuffing"). Similar aggregations of lymphocytes near the pulsed electrodes sites has been seen in the cochlear nucleus when the microelectrodes are pulsed soon (e.g, within 15 days) after implantation. The response was not expected in cat ic144, since the electrodes were pulsed 28 days after implantation. This type of infiltration of inflammatory cells into the neuropil is undesirable, since they may release lymphokines that may encourage formation of scar tissue, or initiate other possible undesirable effects. Since we have never observed such aggregates of lymphocytes near microelectrodes that are pulsed more than 2 months after implantation, we have assumed that it represents some sort of interaction between a cytotropic effect of the electric field or an electrochemical product generated at the electrode-electrolyte interface, and an ongoing inflammatory response to unresolved trauma. In the case of IC144, the unresolved trauma may be associated with the pressure necrosis in the molecular layer as the arrays's spine continued to sink down into the brain.

Cat IC-146.

In this animal, the test stimulation was conducted 33 days after implantation of the silicon array. When this array was received from the University of Michigan, only 8 of the 16 electrode sites were electrically intact. These 8 sites remained functional throughout the 33 days in vivo. Also, the cross-coupling (crosstalk) between any two electrode channels remained below 1% through the time in vivo, indicating no significant incursion of body fluid into the implanted junction box.

At 33 days after implantation, small but usable compound action potentials evoked from at least five stimulating electrodes were recorded from the pyramidal tract. The responses induced by 1,024 to 4,096 stimulus pulses were averaged to generate the

averaged evoked compound action potentials (AECAPS). When acquiring the AECAPS, the stimulus pulses were delivered at a frequency of 20 Hz, and the stimulus pulses were 150 μsec/phase in duration (rather than 400 μsec as was used during the 7-hour test stimulation)

The pyramidal tract response sometimes did not grow monotonically with stimulus amplitude, as illustrated in Figure 3. These responses were evoked from electrode site #10. The amplitude of the stimulus pulses is indicated near the right edge of each trace . Stimulus pulses 16-24 μA in amplitude evoked a large early response, which nearly disappeared when the stimulus amplitude was increased to 30 μA. The short latency of the early response (approximately 1.2 msec after the start of the stimulus pulse) indicates that it was evoked directly, rather than transsynaptically, so it is unlikely that the "quenching" of the response at high stimulus amplitude was due to recruitment of inhibitory synaptic effects. It is likely that this response represents the activity of only a single large cortical neuron which happened to lie close to the stimulating site in the cortex and which also happened to send its axon very close to the recording electrode in the pyramidal tract. In this case, the marked reduction in the amplitude of the average response as the stimulus amplitude increased, may have been due an anodic block on the axon at a site more distant from the stimulating electrode than the site at which the action potentials were initiated .

Figure 4 shows the family of AECAPS induced by electrode #11. In this case, the earliest component of the evoked response was smaller than in Figure 3, but it did increase monotonically with stimulus amplitude, indicating that it is due to the summation of the response of several neurons whose axons do not pass particularly close to the recording electrode. As the stimulus amplitude was increased, the AECAP became more complex, reflecting recruitment of transsynaptically-evoked activity in the corticospinal neurons. We measured the amplitude of the earliest response, which on the basis of its short latency, we assume was evoked directly rather than transsynaptically. The component's amplitude was measured from the peak of the positivity (p) to a baseline connecting the two flanking negativity (n1,n2), as indicated in the figure. The three points

required for this measurement were established from the response evoked by 30 μA where the signal-to-noise ratio was relatively high. Figure 5 shows the recruitment (growth) function of the response before and after the 7-hour test stimulation. Prior to the test stimulation, the threshold of the evoked response was below 10 μA . After the 7-hour test stimulation, the threshold of the evoked response was greater than 20 μA, indicating that marked depression of neuronal excitability had developed during the 7 hours of continuous stimulation..

These chronically-implanted silicon electrodes also allowed recording of the action potentials generated by individual cortical neurons. The signals were recorded by means of an 8-channel system with j-fet head-stages, and digitized into the computer at 25,000 samples/sec. Figure 6 shows the action potentials from 3 or 4 cortical neurons in the motor cortex of cat IC146, 33 days after implantation of the array and before the start of the 7-hour stimulation test regimen. In the future, we plan to use the unitary spike activity as a means of tracking the time-course of any migration of the arrays through the cortex, as discussed below.

During the 7-hour test stimulation, the impedance of two of the 8 electrodes sites became very high, after remaining stable for 33 days in vivo. It is possible that this was due to delamination of the iridium-covered stimulation sites. In the future, we will activate the sites only to a total charge capacity of 6 mC/cm^2, as recommended by the personnel at the University of Michigan. When activated to this charge capacity, only a portion of the sites' iridium over-layer will be converted to hydrous oxide, and the iridium-iridium oxide layer should be less likely to detach from the titanium substrate.

The cat was perfused for histologic evaluation about 90 minutes after the end of the 7-hour test stimulation. During the subsequent autopsy, the array's superstructure was accidently fractured from the probes, which remained in the tissue and had to be retrived with fine forcept. The tissue near probe track #4 apparently sustained some damage as a result of this procedure.

Figure 7A shows a histologic section through the tracks left by the 4 probes in the precruciate cortex (scale =250 μm). The 8 pulsed sites were on tines 3 and 4, which are

in the upper right portion of the photograph. Track #2 passed close to a blood vessel (v) There is an area of tissue rarefaction adjacent to track #1. The rarefied region contains no hemorrhage or neutrophils or lymphocytes that would indicate an acute or chronic inflammatory response to tissue trauma. The damage was probably inflicted after fixation of the tissue, while the silicon probe was being retrieved with fine forceps. The tissue was stained with Masson's Trichrome preparation, which darkly stains connective tissue (scar tissue). Linear glial scars (arrows) are seen extending from the anterior face of each track. No neutrophilic plasma cells and very few lymphocytes are present, and this indicates that there is no ongoing tissue injury. The linear scarring probably occurred earlier, during implantation of the array. Figure 7B shows the linear scar extending from track 3, at higher magnification (scale =50 μm).

Figure 8A shows an oblique section through the track of probe #4, which contained 4 of the pulsed electrode sites (scale =25 μm). Part of the glial sheath surrounding the track had been avulsed during retrieval of the probe. Neurons near the track are flattened, but otherwise appear normal. The adjacent neuropil appear normal. There is no hemorrhage or evidence on ongoing inflammatory reaction. Figure 8B shows the same track at a different depth in the cortex. There is an old glial scar, approximately 150 x 70 μm in cross-section. The scar contains no neutrophils and very few lymphocytes, indicating no ongoing inflammatory tissue response. The scar may be the results of a small hematoma that occurred when the probes were implanted.

Overall, there is no indication of stimulation-induced tissue injury adjacent to any of the probes. Figure 9 depicts the only evidence of an acute inflammatory response that might be attributed to the 7-hour test stimulation (scale =10 μm). Neutrophils (arrows) have begun to emigrate from a blood vessel near the track of one of the probes with pulsed electrode sites.

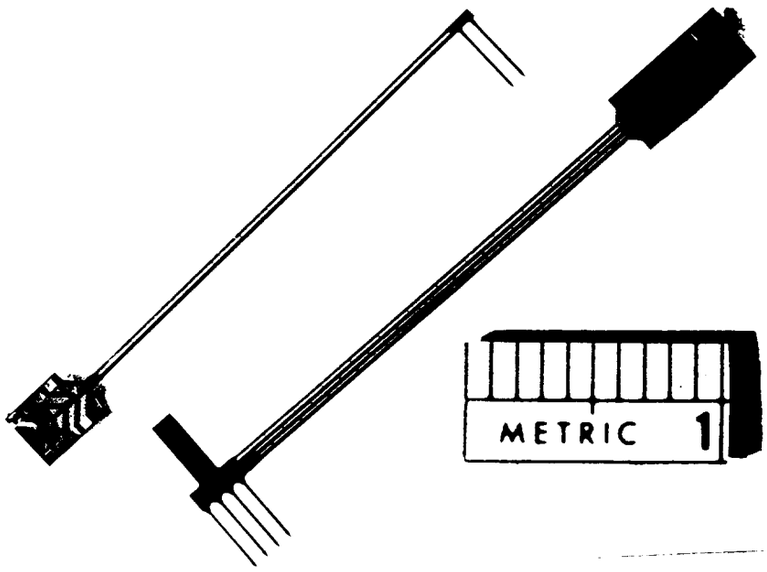
DISCUSSION

We have developed a stimulating microelectrode assembly that incorporates multisite thin film stimulating arrays and have begun chronic implantation of these

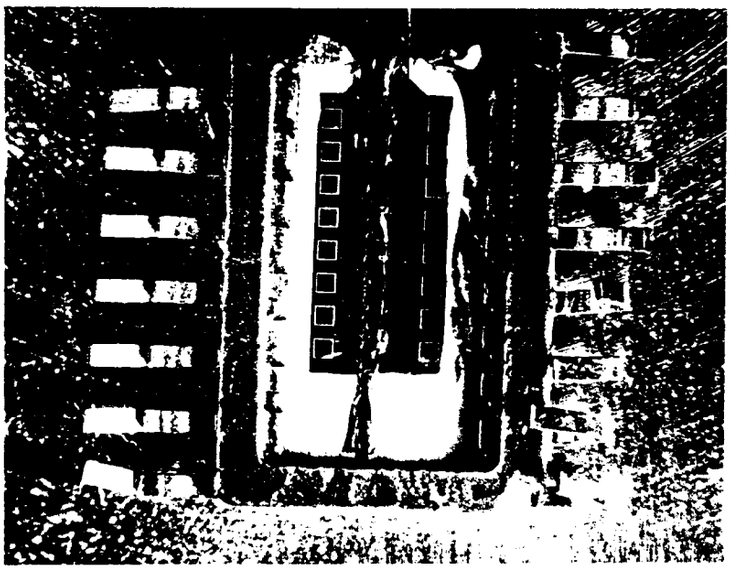
assemblies into the cerebral cortex. The assembly is only adaptable to implant sites within about 2 cm of the bone margin, upon which the rather bulky implantable junction box must anchored. We are now working to resolve some of the remaining problems of implanting these arrays and their long-term residence in the brain. For example, they have a tendency to sink into the cortex and to slash the tissue during implantation. In the histologic evaluations of two experiments conducted to date, tissue injury from mechanical trauma was far more obvious than was injury that might be associated with the electrical stimulation. The primary undesirable consequence of the prolong stimulation (7 hours at 100 Hz, 8 nC/phase) was a marked depression in the electrical excitability of neurons near the stimulating microelectrode. We plan to investigate the effect of interleaved stimulation, and a reduced stimulus duty cycle, on the severity of the depression of neuronal excitability. In the future, we will implant some arrays with the aid of an axial introducer mounted on the stereotaxic frame, and we will determine if this method reduces slashing injury to the tissue.

The problems associated with the electrical stability and integrity of these arrays may be ameliorated by the decision by the personnel at University of Michigan to consign bulk fabrication of the arrays to a commercial chip foundry. We have also modified our procedure for activating (oxidizing) the iridium-coated titanium sites, to ensure that at least some metallic iridium remains below the oxide layer. We hope that this will prevent delamination of the sites during prolonged pulsing.

A



B



C

Figure 1

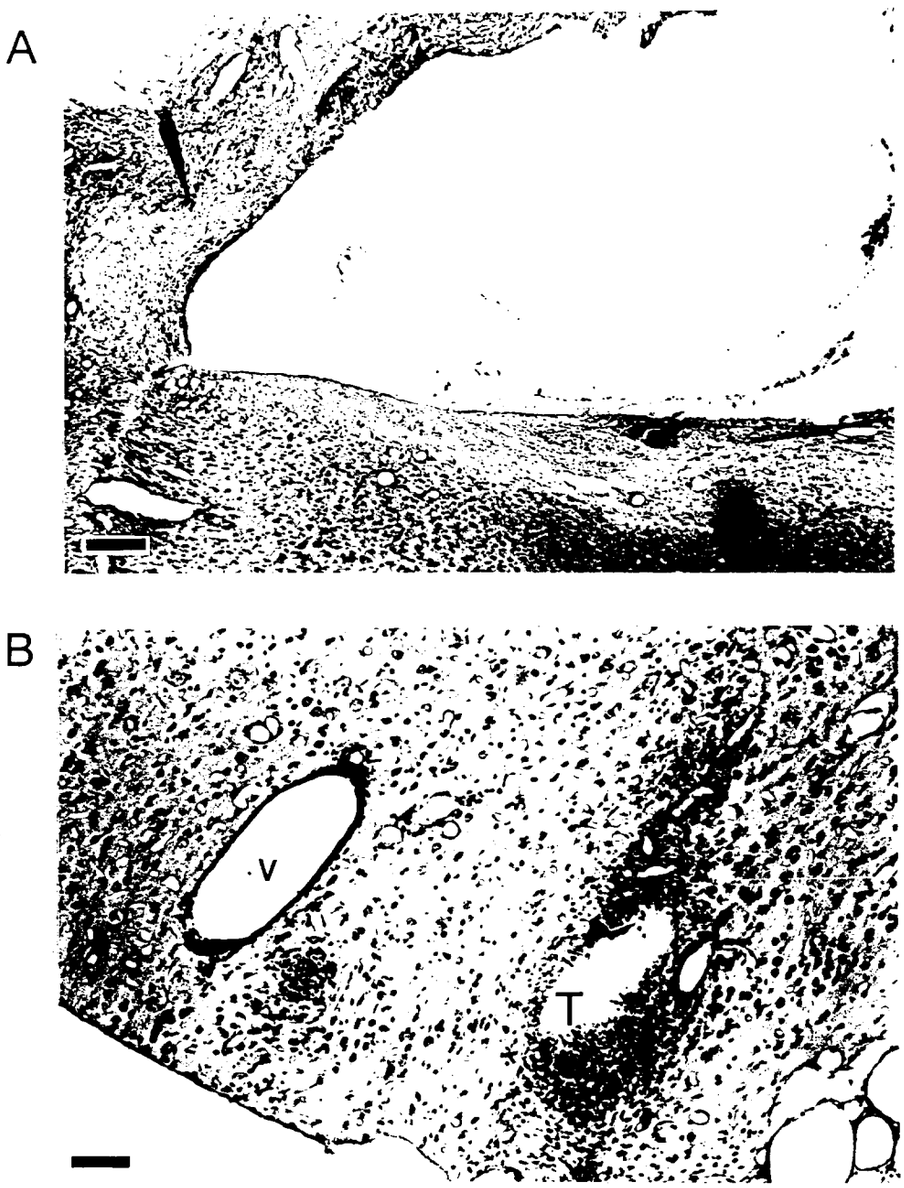
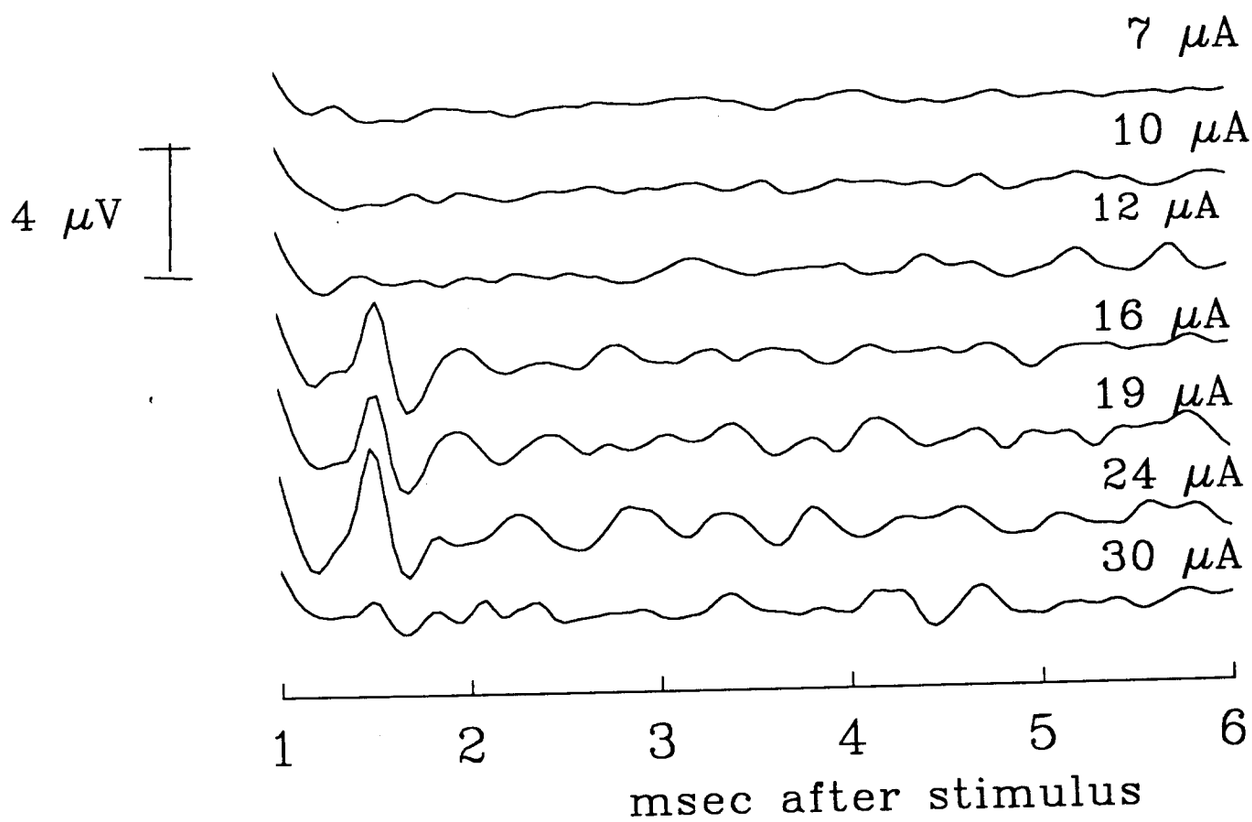


Figure 2

ic146
Electrode #10
Pyramidal tract response 33 days after implantation



ic/icq146l.spg

Figure 3

ic146

Electrode #11, 33 days after implantation

Pyramidal tract response before 7-hour stimulation

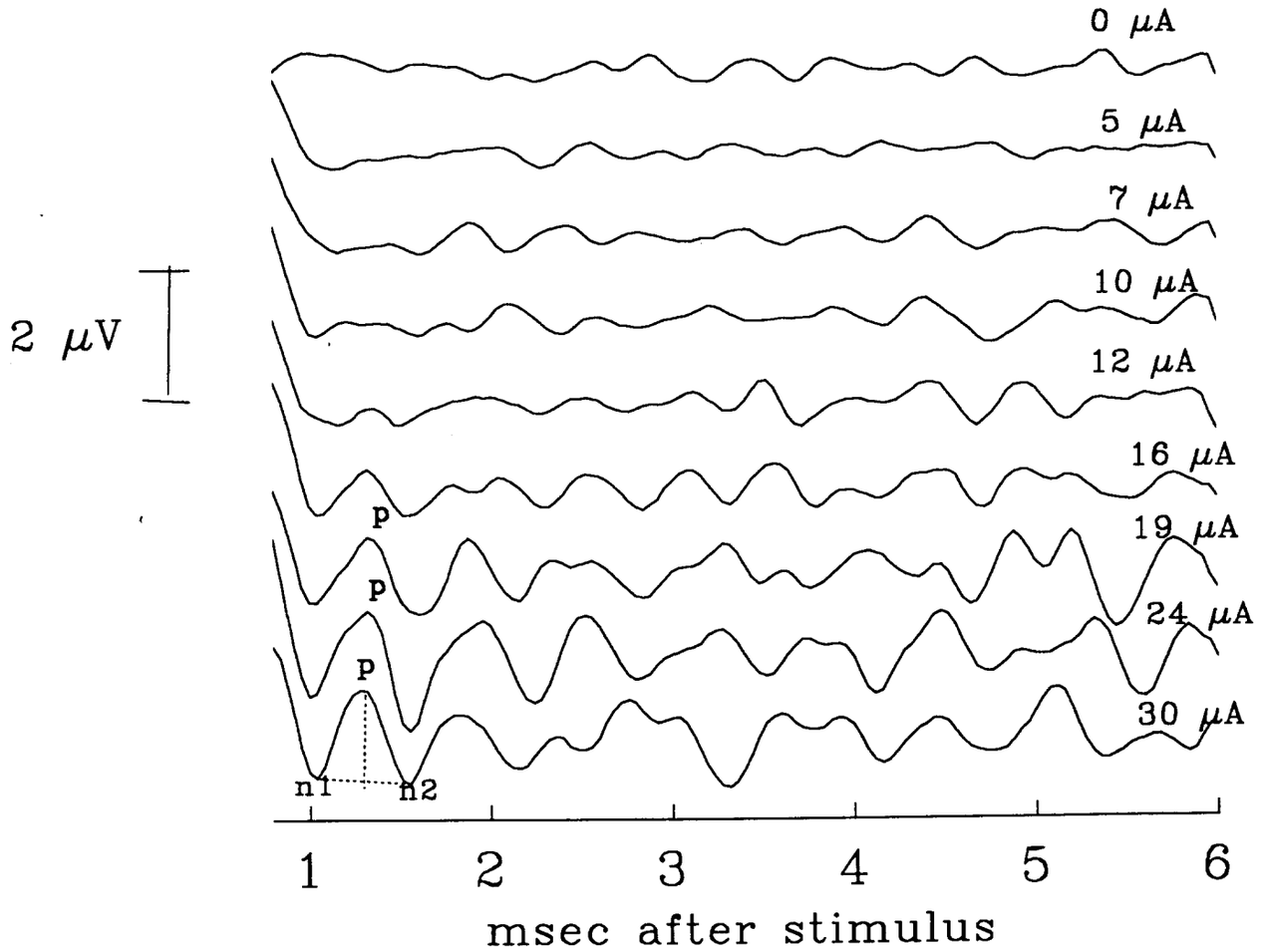


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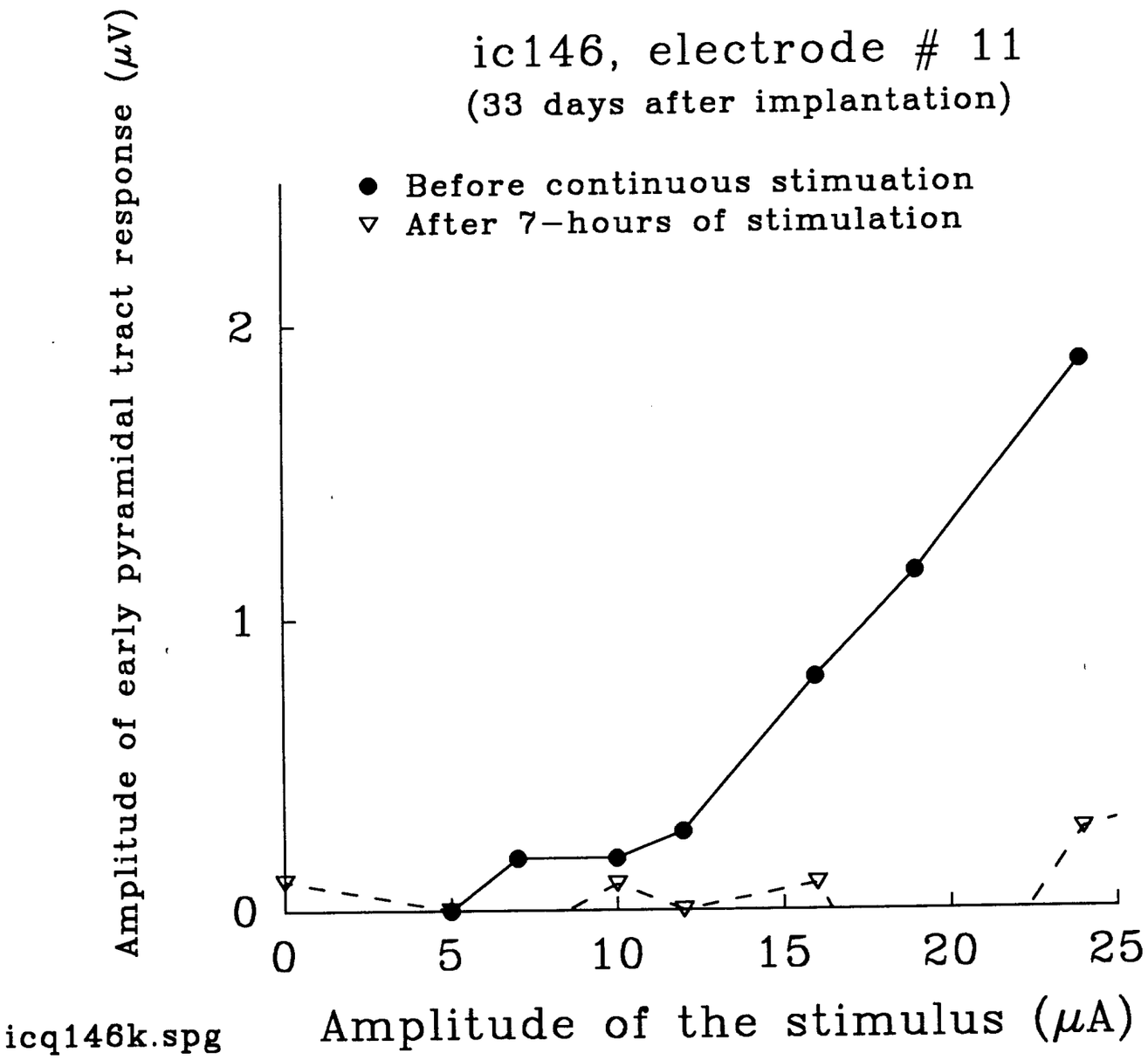
Figure 4



icq146k.spg

Figure 5

ic146 (33 days after implantation)
(Electrode #11)

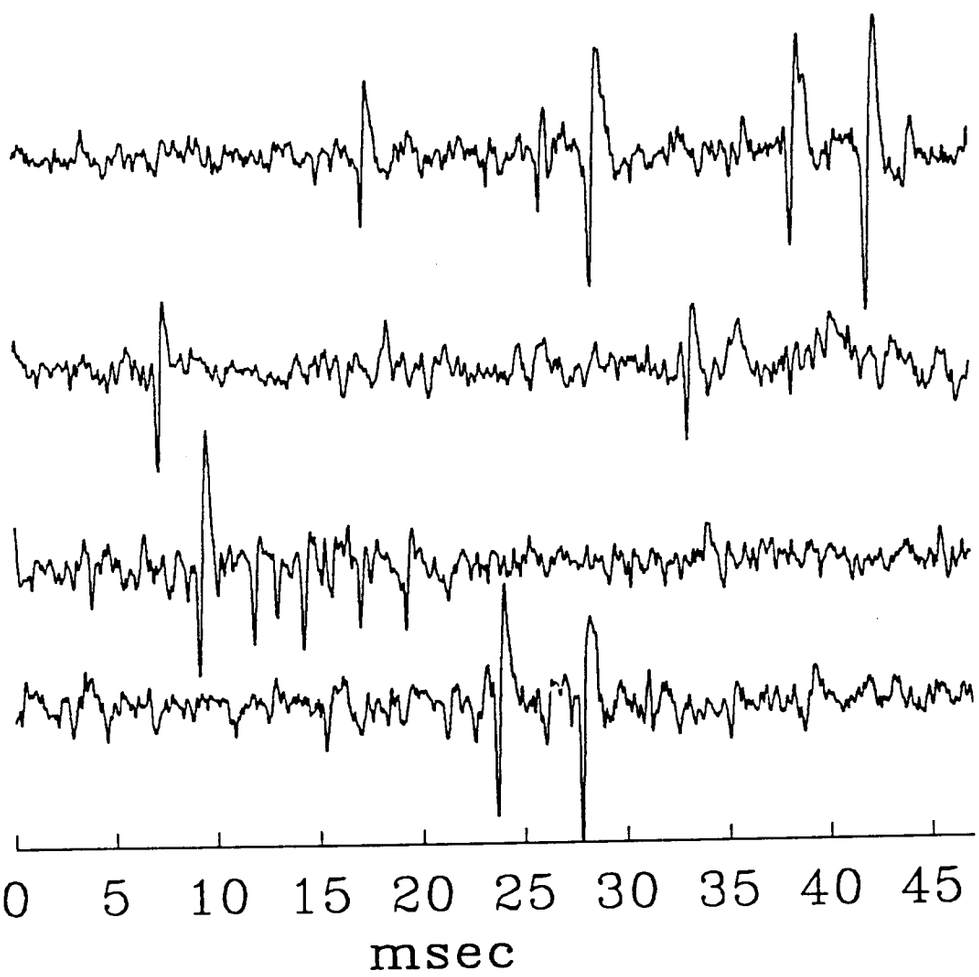


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Figure 6

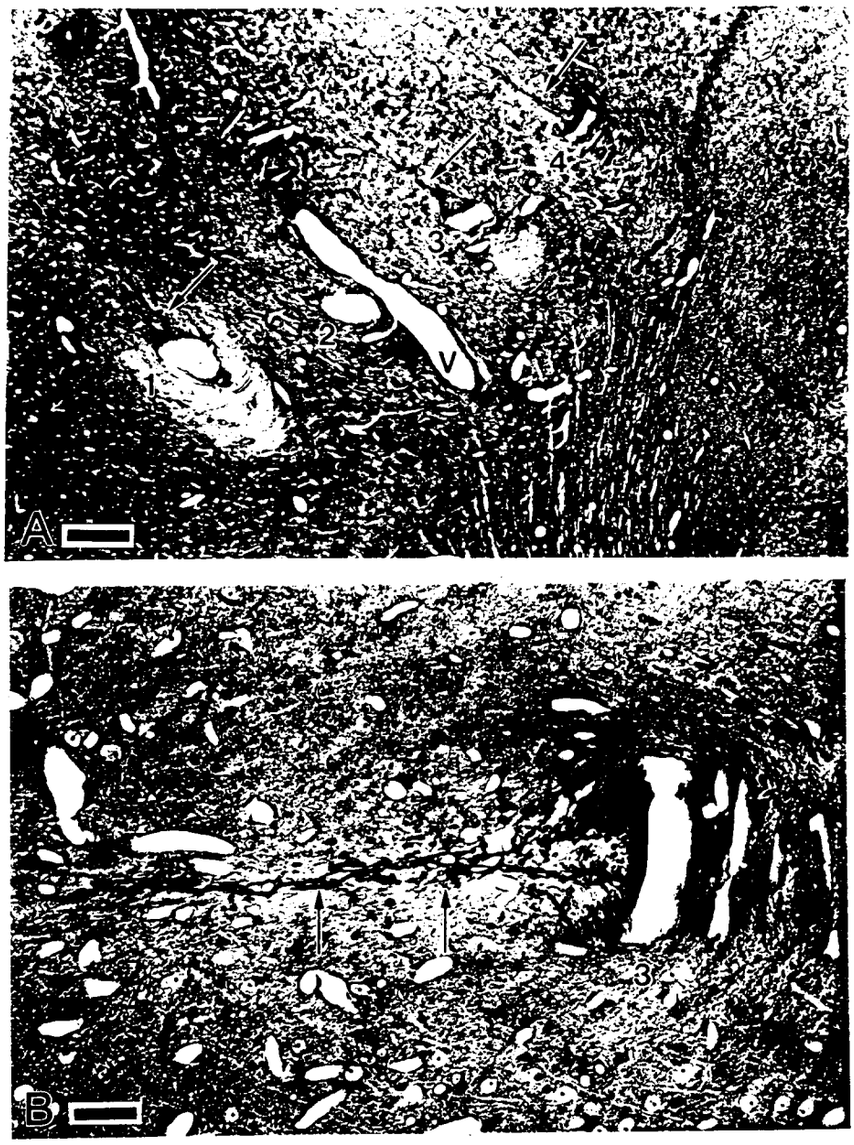

Figure 7

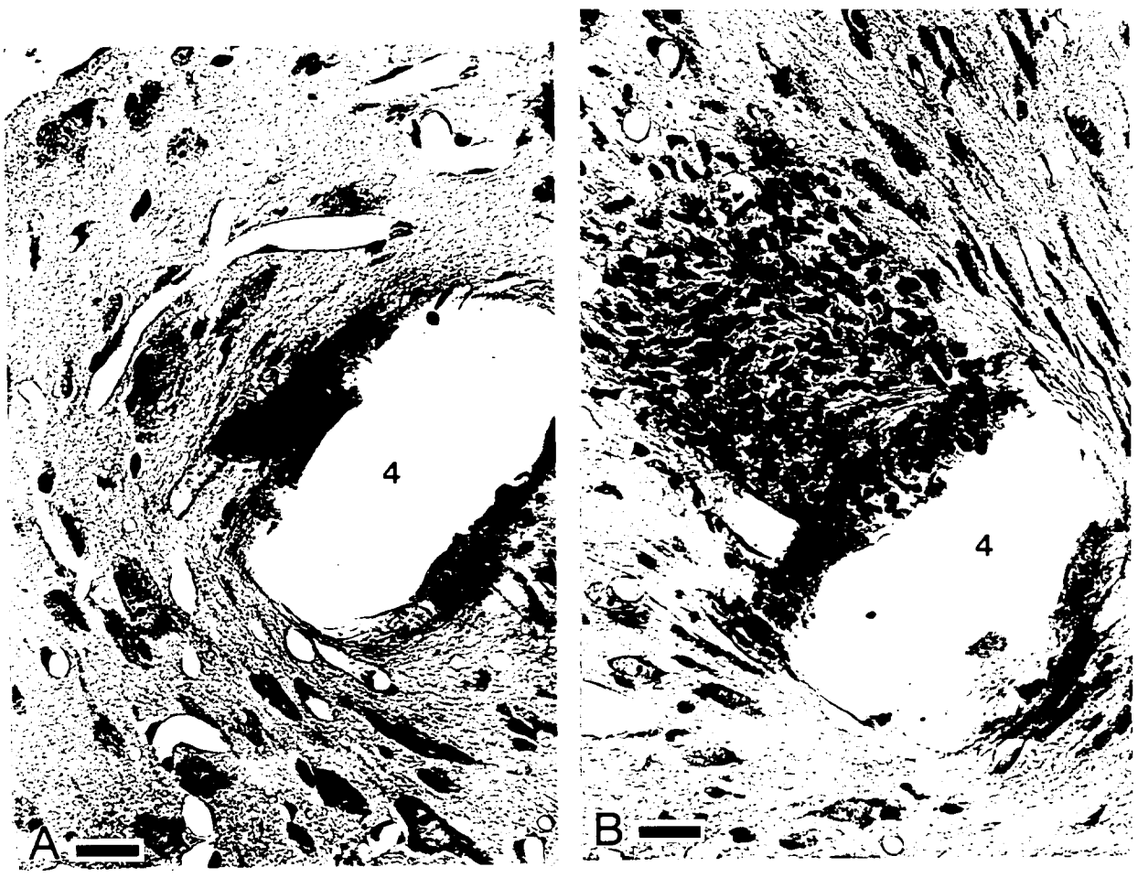

Figure 8

Figure 9